

## WHAT IS CLAIMED IS:

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1. A method for automatically creating crosstalk-corrected data of a microarray, the method comprising:  
 providing a microarray substrate having calibration dye spots, each of the calibration dye spots comprising a single pure dye;  
 for each of the calibration dye spots, generating a dye image containing at least one of the calibration dye spots for each of a plurality of output channels;  
 for each of the calibration dye spots, measuring an output of each of the output channels to obtain output measurements;  
 computing a set of correction factors from the output measurements;  
 and  
 applying the set of correction factors to data obtained from microarray images containing spots having dyes with excitation or emission spectra to obtain crosstalk-corrected data.
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2. The method as claimed in claim 1 wherein the step of generating includes the step of imaging the calibration dye spots to produce a dye image for each calibration dye spot.
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3. The method as claimed in claim 1 wherein the substrate is a glass slide.
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4. The method as claimed in claim 1 wherein each of the channels is optimized for a different dye.
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5. The method as claimed in claim 1 wherein the step of generating is performed by an imager.
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6. The method as claimed in claim 1 wherein each of the dyes is a fluorescent dye.

1                   7.     The method as claimed in claim 1 wherein the step of  
2     computing includes the step of computing crosstalk ratios based on spot brightness  
3     values for each of the calibration dye spots on each of the output channels.

1                   8.     The method as claimed in claim 1 wherein the number of  
2     calibration dye spots is more than or equal to the number of dyes.

1                   9.     The method as claimed in claim 1 wherein the calibration dye  
2     spots are hybridized target DNA and fluorescently labeled probe DNA.

1                   10.    A system for automatically creating crosstalk-corrected data  
2     of a microarray, the system comprising:  
3                   a microarray substrate having calibration dye spots, each of the  
4     calibration dye spots comprising a single pure dye;  
5                   an imager having a plurality of output channels wherein for each of  
6     the calibration dye spots the imager generates a dye image containing at least one of  
7     the calibration dye spots for each of the output channels;  
8                   means for measuring an output of each of the output channels for each  
9     of the calibration dye spots to obtain output measurements;  
10                  means for computing a set of correction factors from the output  
11     measurements; and  
12                  means for applying the set of correction factors to data obtained from  
13     microarray images containing spots having dyes with excitation or emission spectra  
14     to obtain crosstalk-corrected data.

1                   11.    The system as claimed in claim 10 wherein the imager is a  
2     microarray scanner which produces a dye image for each calibration dye spot by  
3     scanning the microarray substrate with a laser of a proper wavelength for the  
4     particular dye.

1                   12.    The system as claimed in claim 10 wherein the substrate is a  
2     glass slide.

1                   13.    The system as claimed in claim 10 wherein each of the  
2 channels is optimized for a different dye.

1                   14.    The system as claimed in claim 11 wherein the microarray  
2 scanner is a confocal laser microarray scanner.

1                   15.    The system as claimed in claim 10 wherein each of the dyes  
2 is a fluorescent dye.

1                   16.    The system as claimed in claim 10 wherein the means for  
2 computing includes means for computing crosstalk ratios based on spot brightness  
3 values for each of the calibration dye spots on each of the output channels.

1                   17.    The system as claimed in claim 10 wherein the number of  
2 calibration dye spots is more than or equal to the number of dyes.

1                   18.    The system as claimed in claim 10 wherein the calibration dye  
2 spots are hybridized target DNA and fluorescently labeled probe DNA.

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